Deacon's Challenge

No 94 - Answer

Serum AFP levels are being monitored following curative surgery for hepatoblastoma in a two-year old boy. Samples are normally being taken at weekly intervals but a repeat sample is taken in error two days after the routine week 4 sample. The requesting clinician is concerned that this sample appears to show evidence of disease recurrence. Assuming a biological variation of 12% and an analytical CV of 6% for this assay, determine whether this concern is justified.

Day	*AFP (kIU/L)*
7	*1,613,000*
14	*723,000*
21	*329,000*
28	*145,000*
30	*149,000*

FRCPath, Autumn 2008

If there had been no recurrence of the tumour then we would expect the AFP to continue to fall after 28 days as it continues to be cleared from the circulation. However, the value at 30 days has risen slightly. So the problem is to decide whether this value is significantly different from the expected concentration taking into account both the biological and analytical imprecision.

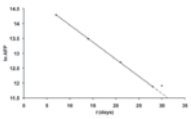
The first step is to estimate the expected AFP concentration at 30 days. The clearance of a tumour marker such as AFP normally follows first-order kinetics and the linear form of the equation is:

$$\ln Cp_t = \ln Cp_0 - k_d.t$$

Where ln Cp_t and ln Cp_0 are the natural logarithms (to the base 2.718, usually denoted as $\log_e$ or ln) of the plasma concentrations at times t and zero respectively. k_d is the elimination rate constant.

A useful first step is to calculate ln for each concentration and to plot the values against t:

Day	AFP (kIU/L)	ln AFP
7	1,613,000	14.29
14	723,000	13.49
21	329,000	12.70
28	145,000	11.88
30	149,000	11.91



The data points for 7, 14, 21 and 28 days all fall on a straight line confirming that the clearance of AFP follows first order kinetics. Furthermore the value at day 30 is above the line suggesting that there may well be a recurrence of the tumour. By extrapolating the line beyond 28 days it is possible to read off the expected ln AFP value at 30 days as 11.66. Taking the antilog then gives a concentration of 116,000 kIU/L (to 3 sig figs).

However, it would be much better to calculate this concentration directly from the data. Using *any two data points* the value of the elimination rate constant (k_d) can be calculated then used to obtain the expected concentration at 30 days. Alternatively, if the day 7 result is taken as the initial value ($\ln Cp_0$), the mean of the results for days 14, 21 and 28 as $\ln Cp_t$ and the mean value of t used, then equal weight is given to each pair of results:

$$\ln Cp_0 = 14.29$$

$$\ln Cp_t = \frac{13.49 + 12.70 + 11.88}{3} = 12.69$$

$$t = \frac{(14-7) + (21-7) + (28-7)}{3} = \frac{7 + 14 + 21}{3} = 14 \text{ days}$$

Substitute these values and solve for k_d:

$$12.69 = 14.29 - k_d.14$$

$$k_d = \frac{14.29 - 12.69}{14} = 0.114 \text{ days}^{-1}$$

Substitution of this value for k_d, the $\ln Cp_0$ result and t corresponding to 30 days (30 – 7 = 23 days) into the rate equation allows calculation of the expected Cp_t:

$$\ln Cp_t = 14.29 - (0.114 \times 23) = 14.29 - 2.62 = 11.67$$

$$Cp_t = \text{antilog } 11.67 = 117{,}000 \text{ kIU/L}$$

which is very close to the graphically determined concentration.

The next step is to calculate the total imprecision at this concentration. The combined CV (CV_{Total}) of this AFP result can be calculated from the expression:

$$CV_{Total}^2 = CV_{Biological}^2 + CV_{Analytical}^2$$

Substitute $CV_{Biological}$ = 12%, $CV_{Analytical}$ = 6%

$$CV_{Total}^2 = 12^2 + 6^2 = 144 + 36 = 180$$

$$CV_{Total} = \sqrt{180} = 13.4\%$$

Use this CV to calculate the standard deviation (SD) at the predicted concentration at 30 days (117,000 kIU/L):

$$CV\,(\%) = \frac{SD \times 100}{\text{Concentration}} \quad \text{so that} \quad SD = \frac{CV(\%) \times \text{Concentration}}{100}$$

Substitute CV = 13.4% and concentration = 117,000 kIU/L:

$$SD = \frac{13.4 \times 117{,}000}{100} = 15{,}700 \text{ kIU/L (3 sig figs)}$$

Therefore the 95% confidence limits at the expected concentration of 117,000kIU/L (mean ±1.96SD) are:

$$117{,}000 - (1.96 \times 15{,}700) \text{ to } 117{,}000 + (1.96 \times 15{,}700)$$

$$= 117{,}000 - 30{,}800 \text{ to } 117{,}000 + 30{,}800 \quad \text{(3 sig figs)}$$

$$= 86{,}200 \text{ kIU/L to } 147{,}800 \text{ kIU/L}$$

The measured value at 30 days (149,000 kIU/L) is just outside of these limits indicating that the clinician's fear of a possible recurrence is justified. The value obtained at the next sampling time (35 days) should clarify.

Question 95

Reproduced below are peak area data from an HPLC analytical run set up to measure plasma phenylalanine. The assay is used to monitor adequacy of dietary control in patients with phenylketonuria, good control being regarded as maintaining plasma phenylalanine between 120 and 360 µmol/L.

N-methyl phenylalanine has been used as the internal standard. 200 µL of internal standard has been added to 200 µL aliquots of samples and standards prior to analysis.

Standard concentration = 500 µmol/L
N-methyl phenylalanine (NMP) concentration = 100 µmol/L
QC target: 180 – 210 µmol/L

Sample	Peak area	
	NMP	Phenylalanine
Standard	20,000	81,000
QC	22,000	35,000
Patient	21,000	140,000

a) Is the assay in control?
b) What is the patient's plasma phenylalanine concentration?
c) What comment would you make about the patient's control from this result?

FRCPath, Autumn 2008

Dear ACB News readers,

I am a Clinical Biochemistry Registrar in Sydney, Australia. Earlier this year I was required to sit a paper of calculations and found the Deacon's Challenge series of problems invaluable in my preparation. However, I believe there is a simple and obvious flaw in the answers to challenge No 84 (March 2008) and a similar problem, No 47 (Feb 2005).

The problems pertain to the question of determining whether a particular concentration of protein in the CSF derives from CSF alone or contains contamination from blood. The solution uses the ratios:

Red cell count in CSF/ red cell count in blood =
Protein concentration in CSF from blood/protein concentration in blood

So far, so good. Except that the figure used in the calculations for protein concentration in blood is in fact protein concentration in SERUM. Obviously these are different values, depending on the haematocrit. I spent hours agonising over this and finally got up the courage to admit to my boss I couldn't understand how a serum concentration could be used instead of blood. I'm passing this on only because he agrees with me and devoted Deacon's Challenge readers might be interested!

Regards

Dr Amanda Caswell
Pacific Laboratory Medicine Services, Royal North Shore Hospital Sydney, Australia